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(54) **IMMOBILIZED POLYETHYLENE OXIDE STAR MOLECULES FOR BIOAPPLICATIONS.**

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(56) References cited :
EP-A- 68 509
EP-A- 263 184
EP-A- 332 261
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WO-A-86/02087
US-A- 4 280 923

(56) References cited :
CHEMICAL ABSTRACTS,vol.113, 08October 1990,(Columbus, Ohio,US), P. Rempp et al:"Anionically polymerized star macromolecules having divinylbenzene cores with grafted poly arms as biomaterials" see abstract 133330f

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Description

Background of the Invention

Polyethylene oxide (PEO) is an important biomaterial because it is non-adsorptive toward biopolymers, and is non-thrombogenic, i.e., it does not adsorb proteins of the intrinsic clotting system nor of the platelet membrane. However, when PEO is combined with other molecules at the surface, thrombogenicity may be enhanced. Okkema, A.Z., J. Biomat. Sci. 1:43-62 (1989). Thus, it is essential that no other molecular entity besides PEO be accessible to proteins. It has been widely studied as a blood-contacting biomaterial in various forms: in segmented polyurethanes, in block copolymers with styrene or siloxane blocks, end-linked into junctions through isocyanate reactions, as side-chains on acrylate polymers and as hydrogels cross-linked from PEO solutions.

PEO is naturally soluble in water and certain organic solvents. Therefore, in order to render PEO insoluble it must be crosslinked, or end-linked to a support. The manner in which this is accomplished often affects physical and chemical properties of PEO.

Chemical crosslinking of PEO can be employed but the chemical crosslinking agent (e.g., a polyglycidoxypropyl siloxane) may be incorporated into the PEO. This can cause adverse biopolymer reactions, including non-specific binding of proteins and platelet adhesion.

Physically crosslinked PEO produced from polyethylene oxide-polystyrene multiblock polymers or from polyether-urethanes suffers from the presence of the non-PEO material at the surface. Adverse biological reactions caused by the non-PEO material can be avoided if the molecular weight of the PEO is made higher than about 5000. However, such material tends to swell excessively in water and is fragile.

End-linking PEO to supports by various means, so as to leave an available hydroxyl group for attachment of an affinity ligand, for example, is not easily carried out if the molecular weight of the PEO is more than about 1000. Furthermore, complete coverage of a surface by end-linking PEO is very difficult, unless the molecular weight is relatively high (several thousand).

Various forms of PEO have also been widely used as a molecular leash for affinity ligands and enzymes. Golander, C.G. et al., Int. Chem. Congress of Pacific Basin Societies, Abstract No. 253, Honolulu, HI, December 17-22, 1989; Harris J.M., J. Macromolecular Sci. C25:325-373 (1985); Holmberg, K., Int. Chem. Congress of Pacific Basin Societies, Abstract No. 255, Honolulu, HI, December 17-22, 1989. Typically, PEO has terminal hydroxyl groups which can be activated for attachment to biopolymers. Most processes for forming PEO biomaterials, however, reduce the hydroxyl content to very low values or zero. In order to produce a crosslinked PEO having a significant concentration of terminal hydroxyls, low molecular weight PEO (2,000 to 10,000) are required but often result in fragile materials. Alternatively, using short PEO side chains on macromonomers like polyethylene glycol methacrylate may result in exposure of the methacrylate residues at the surface.

Thus, a need exists for a method of immobilizing PEO to a support surface without detracting from its physical properties and biological compatability. In addition, it would be desirable to provide a material having a high concentration of hydroxyl groups for attachment to biopolymers.

EP-A-0 156 657 discloses copolymers useful for size exclusion chromatography in aqueous media. The copolymers are partially hydrophilic, non-ionic crosslinked moieties. In particular, they consist of a crosslinked hydrophobic backbone polystyrene of high mechanical strength, with non-ionic hydrophilic chains of polyethylene oxide grafted selectively onto the backbone.

WO-A-8 602 087 teaches a surface-coated article comprising a substrate with a polyethylene oxide based coating thereon. The coating has polyethylene oxide chains with one free end and one end crosslinked to other chains using a special crosslinking agent such as hexamethylenediol diacrylate. Crosslinking is performed by radiation after swelling the substrate.

Chem. Abstr. 133330f, Vol. 113, 08.10.90 concerns biomaterials consisting of anionically polymerized star macro-molecules having divinylbenzene cores with grafted polyethylene oxide arms. This document was published after the Applicant's priority date.

Summary of the Invention

This invention pertains to a method for covalently immobilizing polyethylene oxide star molecules onto a support surface and to hydrogels produced by the method. The PEO star molecules are immobilized in the form of hydrogels using radiation or hydroxyl group activation. The resulting PEO hydrogels have a high concentration of terminal hydroxyl groups which are available for attachment to biospecific affinity ligands or to the support surface itself. As such, the immobilized PEO star molecules can be used as a tool for separating and purifying biological molecules, while greatly reducing or eliminating non-specific binding.

The PEO star molecule hydrogels also have non-thrombogenic properties which make them suitable for applications in which blood contact is required. They are highly biocompatible and have excellent mechanical durability for numerous biomedical applications, including intravenous catheters and implantable vascular prostheses. The hydrogels of this invention can be grafted onto a suitable contact lens material for the manufacture of contact lenses.

The invention will now be described by way of example with reference to the accompanying drawings.

Brief Description of the Drawings

Figure 1a shows a Type I PEO star molecule having a divinyl benzene (DVB) core and PEO chains attached thereto.

Figure 1b shows a Type II PEO star molecule having a DVB core and PEO chains attached thereto by polystyrene (PS) chains.

Figure 2 shows overlapping PEO star molecules (Type I) which are crosslinked to each other by electron irradiation.

Figure 3 shows several PEO star molecules (Type I) covalently attached to a support surface by tresylated hydroxyl groups.

Figure 4 illustrates the attachment of a biopolymer (IgG) to the surface of immobilized PEO star molecules.

Detailed Description of the Invention

Polyethylene oxide star macromolecules have been previously described by Lutz, P. and P. Rempp, Makromol. Chemie 189:1051 (1988) and Gnanou, Y. et al., Makromol. Chemie 189: 2885-2892 (1988) the teachings of which are incorporated by reference herein. The star molecules are synthesized by anionic polymerization from divinyl benzene (DVB), ethylene oxide and optionally styrene. They have a core of divinyl benzene (typically on the order of about 50 angstroms) from which a predetermined number of polyethylene oxide chains or "arms" are grown. The cores however can be of polymeric material other than divinyl benzene. The length of each PEO chain corresponds to its molecular weight and typically range from about 1,000 to about 10,000. Preferably, each star molecule will have from about 6 to about 50 arms. Two variations of PEO star molecules are shown in Figures 1A and 1B and are described herein as Type I and Type II, respectively. Type I star molecules contain a plurality of hydroxyl-terminated PEO chains (hydrophilic) that are attached to a hydrophobic DVB core by non-hydrolysable carbon-carbon bonds. Type II PEO star molecules are of similar composition except that the PEO chains are attached to the DVB core via hydrophobic polystyrene (PS) chains.

The concentration of hydroxy-termini on the PEO arms can be determined in advance by selection of the gross concentration of star molecules and the number of arms carried by the molecule. For example, a star molecule of 100,000 molecular weight with 20 PEO arms has 20 hydroxyls. To obtain comparable hydroxyl concentrations with linear PEO polymers, the molecular weight would have to be lowered to 10,000. However, hydrogels made of cross-linked linear PEO of comparable molecular weight (MW 10,000) are very fragile.

The PEO star molecules can be immobilized or grafted onto a support surface of any geometry (e.g., particles, porous plastic cores, thin plastic film, biomedical device, contact lenses) using ionizing radiation. According to the method, PEO star molecules are dissolved or suspended in an aqueous solution (preferably water) in a concentration sufficient to provide enough star molecules to cover the support surface to desired thickness. Typically, a sufficient concentration will be around 5 to 15 wt/vol%. Type I star molecules form optically clear homogeneous solutions in water, while Type II star molecules form faintly turbid to opaque suspensions, due to the presence of polystyrene. The resulting solution is then deposited onto the support surface, such as by spreading, rotating the support or centrifugation.

The star molecules are then crosslinked together by exposing them to electron beam radiation which results in the formation of a hydrogel network. The term "hydrogel" refers to a broad class of polymeric materials which are swollen extensively in water but which do not dissolve in water. Typically, the solution is exposed to electron radiation in the range of from about 1 to about 6 megarads, most preferably 4 megarads. Gamma radiation can be used as the radiation source but may result in the degradation of the star molecules. Crosslinking occurs randomly between segments of the PEO arms, thus allowing the terminal hydroxyl groups to remain available for subsequent activation, such as coupling affinity ligands to the PEO arms.

Figure 2 shows several Type I PEO star molecules crosslinked together by electron radiation. The resulting hydrogel layers are of variable thickness but are typically on the order of magnitude of >1μM. The thickness of the hydrogel layer can be regulated by various techniques, such as doctor-blade spreading on a support web or centrifugal casting in tubes.

An advantage of electron radiation crosslinking is that the crosslinking reaction proceeds very rapidly, at

a rate of approximately 1 foot/sec. in the case of web coating. The reaction proceeds by free-radical coupling to produce a pure product. As such, the crosslinking reaction does not alter the chemical composition of the star molecules. Other known crosslinking techniques tend to introduce chemical components which may subsequently affect its biocompatibility. Further, the hydrogel network has a surface for contacting biological materials (eg. blood) which is essentially PEO chains. As such, the DVB and PS components are inaccessible or not recognizable to these biological molecules.

The resulting hydrogels have significantly greater mechanical strength than hydrogels formed from ordinary linear PEO having the same range of molecular weight as the star (i.e., 100,000 to 300,000). A gel made from 10 wt.% of 100,000 molecular weight linear PEO under identical dosage would have 2 to 10 times lower tensile strength than the network formed from star molecules, and would have only 1/10th the number of hydroxyl groups per unit area of surface. The concentrations of hydroxyl ends obtained by stars would translate to linear polyethylene oxide of around 5,000 mol. wt. or less. Such low molecular weight polymers cannot be crosslinked at all, or form gels of low strength with considerable soluble fraction.

In another embodiment, the star molecules can be covalently immobilized to a support surface by tresylation of the terminal hydroxyl groups. The support surface and star molecules are each pretreated prior to immobilization. As such, the support surface should contain active functional groups for immobilizing tresylated star molecules thereto, such as amino and/or thiol groups. Likewise, the star molecules should be tresylated in an appropriate solvent at pH 10 or above, prior contacting with the support surface. Tresylation is particularly convenient since the PEO is solvated by media appropriate to tresyl chloride (e.g., dichloromethane, chloroform). This method results in a mono-layer coating of the hydrogel over the support surface.

According to this method, an organic solution comprising PEO star molecules is exposed to tresyl chloride, under conditions such as to fix the tresyl groups to hydroxyl-termini on the star molecules. The resulting tresylated PEO star molecules are then transferred from the organic solvent to an aqueous solution. The pH of the aqueous solution is then adjusted to about 10 or above, so as to favor reaction with amino and/or thiol groups on the support surface. The pH-adjusted solution is contacted with a pretreated surface support that contains amino and/or thiol groups, under conditions whereby the star molecules become covalently bound in a dense layer to the support surface.

This process is further described below by way of illustration. For example, a Cellophane™ (cellulose containing plasticizers) containing support is placed in a bath of tetrahydrofuran and tresyl chloride. The hydroxyl groups on the surface of the Cellophane™ are then tresylated. Once tresylated, the Cellophane™ is aminated in a water solution of mercaptoethanol amine (pH 10) which results in binding the group $-SCH_2CH_2NH_2$ to the activated hydroxyl groups. Likewise, star molecules are tresylated and then placed into an aqueous buffer (pH 10) containing the aminated Cellophane™. After a period of time (approx. 1 hr), the Cellophane™ is removed from the solution and rinsed to wash off any unbound star molecules. The star molecules become bound to the amino group via the tresylated hydroxyls. Figure 3 shows several PEO star molecules immobilized on a support surface. The attachment results from the reaction of amino groups on the support surface with tresylated hydroxyls on the star molecules.

The star molecule hydrogels can be covalently bonded onto an appropriate support surface using the methods previously described to thereby protect the support from recognition by biopolymers. A monolayer coating of PEO star molecules can be accomplished by attaching one or more PEO arms to the support. The remaining arms remain available for attaching biopolymers or affinity ligands. The PEO-coated support surface can then be exposed to a biopolymer having amino or thiol groups which can couple to available tresylated hydroxyl groups. These available groups function as molecular leashes or tethers for the biopolymer. For example, anti-Protein C antibody can be attached to the star molecules and will be selective for its antigen, Protein C. The PEO monolayer prevents adsorption of the biopolymers onto the support surface and can thereby reduce or eliminate non-specific binding of undesired biopolymers. Figure 4 demonstrates the use of star molecules for attaching affinity ligands, such as Immunoglobulin G. The symbol ♦ represents a covalent linkage between a PEO arm and an amino group on the support; • represents a covalent linkage between a PEO arm and an amino or thiol group on IgG; ★ represents an endcapped previously tresylated hydroxyl (e.g., by treatment with mercaptoethanol).

Due to the number of available PEO arms which can accommodate ligands, the hydrogels of this invention can be used to continuously separate, purify and concentrate therapeutic proteins. Processing of the proteins will require cycles of coupling and decoupling of the ligate to affinity ligands bound to the stars.

The affinity surface can be of any geometric shape, such as particles packed in beds, freely moving particles and porous membranes. The hydrogels can be coated onto silica particles. In this case, polyethylene oxide is physically adsorbed to the silica surface but cannot be covalently bound unless the silica has been previously modified. Nonetheless, the polyethylene oxide hydrogel forms a shell covering the particle and it

thus cannot escape. The hydrogels can also be deposited into pores of ultrahigh molecular weight, high density polyethylene such as Porex™ (Auburn, Georgia), on the surface of Coretex™ e-PTFE (expanded polytetrafluoroethylene) and Mylar™ film.

In most cases, once a PEO hydrogel is coated onto the affinity surface, the terminal hydroxyl groups are activated by tresylation. Preferably, this is accomplished by contacting the hydrogel with tresyl chloride dissolved in an organic solvent, such as dichloromethane. The tresylated PEO star molecules are then placed in buffered aqueous solution containing the affinity ligand which is to be bound. Examples of preferred ligands include antibodies and F_{ab} fragments thereof, Protein A, active polysaccharides, heparin-NH_2, anti-Protein C IgG, and the F_{ab} fragment of anti-Protein C IgG.

Following affinity bonding of a specific ligate to its bound ligand, the hydrogel-coated affinity support is washed to remove unbound proteins. Remaining bound proteins are then decoupled by changing the composition of the eluting buffer, for example by changing the ionic strength or the pH (e.g., to pH 10 or above) of the eluting buffer. For example, a 1 M NaCl decoupling solution can be used in the case of antithrombin III bound to heparin. The decoupling results in free ligate in the eluting buffer. The ligate can then be separated from the eluting buffer using known techniques, such as by diafiltration described by Herak and Merrill, Biotech. Prog. 5:9-17(1989). Separated ligates can then be concentrated using known techniques. Examples of some specific ligates include macromolecules, monoclonal antibodies, antigens, viruses and cells(e.g., blood platelets, white blood cells, endothelial cells and other non-blood cells).

In addition to bioseparations, the hydrogels made according to this invention are useful for a variety of biomedical applications, due to their non-thrombogenic properties and excellent mechanical durability. They are suitable for in vivo applications in which blood contact is required, including blood contacting implantable vascular prostheses, angioplastic stents, cardiovascular sutures, metabolic support catheters , angioplastic balloon catheters, intraaortic balloon pumps, pulmonary artery catheters, artificial hearts and ventricular assist devices. The hydrogels may also be used for ex vivo devices, such as hemodialysis membranes and membranes for extra-corporeal oxygenators.

A preferred application for the star molecules of this invention is in the manufacture of contact lenses. PEO star molecules can be grafted onto a suitable art recognized contact lens material, such as gas permeable PEO, using the techniques described herein. For example, the contact lens material can be immersed in a PEO star molecule solution and exposed to ionizing radiation to thereby graft the star molecules onto the contact lens surface. Alternatively, the surface of the contact lens material can be modified by creating amino or thiol groups on its surface. The modified lens material is then exposed to activated PEO star molecules, such as tresylated star molecules described above. Due to the properties of the star molecules, absorption of proteineous deposits from natural enzymatic secretions of the eye by the star molecule coated-contact lens material can be eliminated or substantially reduced. Thus, the coated lenses will not become clouded or opaque because of lowered protein absorption.

Additional chemical components can be incorporated into the star hydrogels depending upon the application. In some instances it may be advantageous to incorporate heparin into the hydrogel to further reduce thromogenicity. While heparin can be attached covalently to tresylated hydroxyls on the star molecules, it is also readily incorporated at high concentrations in the hydrogel by simply adding it to the solution of the star before irradiation. In this form it elutes into the blood flow over a significant period of time.

The invention will be further illustrated by the following Example.

Exemplification

Synthesis and Characterization of Various PEO Hydrogels

Linear PEO and various forms of star molecules having the physical properties described below were electron beam irradiated, at a dose rate of about 0.1 megarad per second, and with a 2 megarad dose per pass under the beam to form hydrogels. Radiation was delivered from a 3 MeV Van de Graaff generator (MIT High Voltage Research Laboratory).

Table 1 presents the apparent swelling ratio q at 25°C (q = volume of hydrogel equilibrated in water/volume of original mixture irradiated) as a function of radiation dose D in megarad, and as a function of the star type. Two linear PEO samples are included for reference. The concentration of the solution as irradiated in every case was 10.0 wt/vol.% in MilliQ^R water. From Table 1 it is apparent that the swelling ratio q of hydrogels formed from star molecules is significantly less than for hydrogels from linear PEO types. Furthermore, the high styrene content Type II hydrogels (3103, 3229) exhibit virtually no swelling.

TABLE 1

Swelling Ratios q of 10 wt/vol.% Polymer/Water After Electron Beam Irradiation

Linear PEO	D	q	[OH] μM
Nominal 300,000 m.w.	4	2.03	0.33
	6	1.92	0.35
Nominal 100,000 m.w.	4	2.8	0.71
	6	2.4	0.83

Type I Stars (no styrene)

	mol. wt. total	# arms	M_{PEO}	D	q	[OH] μM
3098	229,000	43	5300	4	1.3	14.6
3210	142.000	40	3460	4	1.4	20.0
3224	79,000	8	10,000	6	1.6	6.3

Type II Stars

	mol. wt. total	%S	#arms	M_{PEO}	M_{PS}	D	q	[OH]
3103	190,000	20	16	8000	2000	4	~1.0	8.4 μM
3229	257,000	30	25	6800	3200	4	~1.0	9.6
3385	371,000	2	30	12,000	520	4	1.7	4.7
						6	1.6	5.7

D: dose in megarads
Total mol. wt. of stars by light scattering
q: Swelling Ratio
[OH]: g. equiv. per liter of gel swollen to equilibrium in water at 25°C.

From the results, the random cross-linking of star molecules cannot be expected to lead to networks like those produced from randomly cross-linked linear macromolecules, in which the functionality of the junction ϕ is necessarily 4. In contrast, the incorporation of stars implies incorporation of junctions of high functionality ϕ, i.e., ϕ = # arms. Further, the "junction" is in effect a high modulus poly DVB core, in Type I stars, and an even more complicated entity, i.e., poly DVB with short polystyrene arms, in Type II stars. Thus, the space occupied by the "junction", and the thermodynamically adverse junction-water interaction place the star hydrogel beyond the tenets of the Flory-Huggins theory of swelling of randomly crosslinked networks.

The last column in Table 1 shows the molar hydroxyl content of the gel at equilibrium in water [OH], calculated as: (mols OH/100 g. dry polymer)q^{-1}, wherein the first term is determined as (number of arms/total mol. wt.).100. Each original solution at 10 wt/vol.% contains 100 g dry polymer per liter. The final wt/vol.% polymer in the get at equilibrium with water is thus 10/q. This is very important if the star hydrogel is to be deployed as a model biomaterial to which bioactive species are to be grafted. It is desirable to have a high value of [OH] and a low swelling ratio q in order that the biomaterial remain approximately in the shape in which it was cast. Stars 3098 and 3210 as hydrogels provide examples.

In the hydrated state, i.e., in equilibrium with blood plasma, preliminary studies of platelet deposition indicate that the surface of star hydrogel is entirely PEO, that is, the poly DVB core is buried and inaccessible, because of the fact that the Star hydrogel acts as if it were a hydrogel of linear PEO. Crosslinking of these

arms is random. Granting that all PEO arms have approximately the same molecular weight on a given star type as a consequence of the anionic polymerization route. Under an electron beam hydroxyl radicals created from water constitute the principal reagent and therefore the PEO rather than the poly DVB and PS experiences macroradical formation and subsequent coupling. To some degree scission of the arms must occur competitively with cross-linking under radiation. The terminal hydroxyl concentrations [OH] calculated in Table 1 do not take this into account.

Biocompatability

Hydrogels containing Type I Stars 3098 or Type II Stars 3385, described above, were examined for biocompatability.

Tubular specimens of hydrogel were prepared from 10 wt/vol.% solutions of star polymers 3098 and 3385 using 0.7 ml of solution centrifugally cast and irradiated under 6 megarads inside glass tubes of 10 cm length x 9 mm lumen. These were tested in an ex vivo shunt model [indium 111 labeled platelets, baboon] with uncoated glass tubes as control. Over a period of 1 hour at a blood flow rate of 100 ml/min., there was no increase of indium count above background for the two hydrogel surfaces, whereas in glass control tubes (no coating) the count more than trebled over background.

Using similar techniques, glass tubes lined with 0.7 ml hydrogels formed from 10 wt./vol. % solutions of linear PEO of 100,000 and 300,000 mol. wt., respectively, under the same dose were prepared. Upon equilibration at 25°C with pure water, the apparent swelling ratios (final volume:initial volume) were : 1.3, 1.3, 2.8 and 2.0 for Star 3098, Star 3385, PEO 100,000 and PEO 300,000 hydrogels, respectively. Values of 1.3 as compared to 2 or more mean that the star polymer based hydrogels when exposed to blood do not expand to such a degree as to compromise attachment to the surface on which they were cast. The lack of platelet uptake indicates that the star polymers in hydrogel form present a "pure" PEO surface to blood. As such, the DVB cores were shielded from access of plasma proteins by the PEO arms.

Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following Claims.

Claims

1. A method of immobilizing polyethylene oxide star molecules to a support surface in the form of a hydrogel, comprising the steps of:
   a) providing a solution comprising polyethylene oxide star molecules having a plurality of hydroxy-terminated polyethylene oxide chains attached to a polymeric core;
   b) depositing the solution onto a support surface; and
   c) immobilizing the star molecules to the support surface in the form of a hydrogel.

2. The method of Claim 1, wherein step c) is performed by irradiating (e.g. electron beam radiation) the solution to produce a hydrogel of crosslinked star molecules, wherein the solution is an aqueous solution.

3. The method of Claim 1, further comprising the step of providing tresylated star molecules in an aqueous solution at a pH of above 10 prior to step b), and wherein the support surface contains active functional groups (e.g. thiol, amino or both) for immobilizing the tresylated star molecules thereto.

4. The method of Claim 1, 2 or 3, wherein the polymeric core is divinyl benzene.

5. The method of any one of Claims 1 to 4, wherein the support surface is selected from particles, porous polymeric membranes, polymeric films, ultrahigh molecular weight high density polyethylene and biomedical devices.

6. The method of any one of Claims 1 to 4, wherein the support surface is selected from blood contacting vascular prostheses, angioplastic stents, cardiovascular suture, metabolic support catheters, angioplastic balloon catheters, artificial hearts, ventricular assist devices, hemodialysis membranes and mem-

branes extracorporeal oxygenators.

7. A method of Claim 3, comprising the steps of:
(i) exposing an organic solution, which contains polyethylene oxide star molecules having a plurality of hydroxy-terminated polyethylene oxide chains attached to a divinyl benzene core, to tresyl chloride to fix tresyl groups to the hydroxy termini;
(ii) transferring the tresylated polyethylene oxide star molecules from the organic solvent to an aqueous solution;
(iii) adjusting the pH of the aqueous solution to 10 or above; and
(iv) contacting the solution of step (iii) with a support surface containing amino or thiol groups or both for immobilizing the tresylated star molecules thereto, whereby the star molecules are covalently bound in a dense layer to the support surface.

8. The method of Claim 7, further comprising the steps of:
a) washing the support surface to remove any non-bound star molecules, leaving the tresylated polyethylene oxide star molecules remaining bound thereto; and
b) contacting the support surface after step a) with an affinity ligand of interest having amino or thiol groups or both thereon, to bind the ligand to the polyethylene oxide chains.

9. The method of Claim 8, wherein the affinity ligand is selected from antibodies, Protein A, F_{ab} fragments of antibodies and active polysaccharides (e.g. heparin).

10. A product produced by the method of any one of Claims 1 to 9.

11. A method of separating and purifying a ligate of interest, comprising the steps of:
a) providing a support surface having coated thereon, a hydrogel comprising polyethylene oxide star molecules having a plurality of ligand-terminated polyethylene oxide chains attached to a divinyl benzene core;
b) contacting a sample containing a ligate of interest under conditions sufficient to bind the ligate to the ligand;
c) removing any unbound proteins from the hydrogel-coated surface;
d) adjusting ionic strength of the sample to thereby remove the bound ligate from the hydrogel; and
e) collecting the separated ligates.

12. The method of Claim 11, wherein the support surface is selected from silica particles, porous polymeric material, polymeric film and ultrahigh molecular weight high density polyethylene.

13. The method of Claim 11 or Claim 12, wherein the ligate is selected from macro-molecules, monoclonal antibodies, antigens, viruses and cells (e.g. blood platelets, white blood cells and endothelial cells).

14. The method of Claim 11 or Claim 12, wherein the ligand is selected from antibodies (e.g. monoclonal anti-Protein C IgG or F_{ab} fragments of monoclonal anti-Protein C IgG), Protein A, F_{ab} fragments of antibodies, and active polysaccharides (e.g. heparin).

15. A biocompatible, non-thrombogenic hydrogel consisting essentially of crosslinked polyethylene oxide star molecules, which are immobilized to a support surface, said molecules having a plurality of hydroxy-terminated polyethylene oxide chains attached to a polymeric core.

16. The hydrogel of Claim 15, wherein the star molecules comprise hydroxy-terminated polyethylene oxide chains attached to a divinyl benzene polymeric core, wherein each chain has a molecular weight range of from about 1,000 to about 10,000.

17. A contact lens comprising the hydrogel of Claim 15.

18. A contact lens of Claim 17, wherein the polymeric core is divinyl benzene and each chain has a molecular weight which ranges from 1,000 to 10,000.

Patentansprüche

1. Methode zur Immobilisation von Polyethylenoxid - Sternmolekülen in Form eines Hydrogels auf einer Trägerfläche, die folgende Schritte umfaßt:
   a) Bereitstellung einer Lösung, die Polyethylenoxid - Sternmoleküle mit einer Vielzahl von hydroxyendständigen, an einen polymeren Kern gebundenen Polyethylenoxidketten enthält;
   b) Auftragen der Lösung auf einer Trägerfläche; und
   c) Immobilisation der Sternmoleküle auf der Trägerfläche in Form eines Hydrogels.

2. Methode gemäß Anspruch 1, dadurch gekennzeichnet, daß Schritt c) durch Bestrahlung der Lösung, z. B. Elektronenbestrahlung, zur Erzeugung eines Hydrogels aus vernetzten Sternmolekülen erfolgt, wobei die Lösung eine wäßrige Lösung ist.

3. Methode gemäß Anspruch 1, die vor Schritt b) als weiteren Schritt die Bereitstellung tresylierter Sternmoleküle in einer wäßrigen Lösung mit einem pH Wert von über 10 aufweist und dadurch gekennzeichnet ist, daß die Trägerfläche aktive, funktionelle Gruppen, z. B. Thiol-, Amino- oder beide enthält, um darauf tresylierte Sternmoleküle zu immobilisieren.

4. Methode gemäß Anspruch 1, 2 oder 3, dadurch gekennzeichnet, daß der polymere Kern Divinylbenzol ist.

5. Methode gemäß einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, daß die Trägerfläche aus Partikeln, porösen, polymeren Membranen, polymeren Filmen, ultrahochmolekularem Polyethylen mit hoher Dichte und biomedizinischen Hilfsmitteln ausgewählt ist.

6. Methode gemäß einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, daß die Trägerfläche aus blutkontaktierenden Gefäßprothesen, angioplastischen Stents, kardiovaskulärem Nahtmaterial, stoffwechselunterstützenden Kathetern, angioplastischen Ballonkathetern, künstlichen Herzen, ventrikulären Hilfsmitteln, Hämodialysemembranen und Membranen extrakorporaler Sauerstoffgeräte ausgewählt ist.

7. Methode gemäß Anspruch 3, die folgende Schritte umfaßt:
   (i) Behandlung einer organischen Lösung, die Polyethylenoxid - Sternmoleküle mit einer Vielzahl hydroxyendständiger, an einen polymeren Kern gebundener Polyethylenoxidketten enthält, mit Tresylchlorid, um Tresylgruppen an den Hydroxyenden zu fixieren;
   (ii) Überführen der tresylierten Polyethylenoxid - Sternmoleküle vom organischen Lösemittel in eine wäßrige Lösung;
   (iii) Einstellen des pH Werts der wäßrigen Lösung auf 10 oder höher; und
   (iv) Kontaktierung der Lösung aus Schritt (iii) mit einer Trägerfläche, die Amino- oder Thiolgruppen oder beide enthält, um darauf tresylierte Sternmoleküle zu immobilisieren, wodurch die Sternmoleküle kovalent in einer dichten Schicht an die Trägerfläche gebunden werden.

8. Methode gemäß Anspruch 7, die außerdem folgende Schritte umfaßt:
   (a) Waschen der Trägerfläche zwecks Entfernung aller ungebundenen Sternmoleküle, wodurch die tresylierten Polyethylenoxid - Sternmoleküle darauf gebunden zurückbleiben; und
   (b) Kontaktierung der Trägerfläche im Anschluß an Schritt a) mit dem betreffenden affinen Liganden, der Amino- oder Thiolgruppen oder beide aufweist, zwecks Bindung des Liganden an die Polyethylenoxidketten.

9. Methode gemäß Anspruch 8, dadurch gekennzeichnet, daß der affine Ligand aus Antikörpern, Protein A, F_{ab} - Fragmenten von Antikörpern und aktiven Polysacchariden, z. B. Heparin, ausgewählt ist.

10. Ein nach der Methode gemäß einem der Ansprüche 1 bis 9 hergestelltes Produkt.

11. Methode zur Abtrennung und Reinigung eines in Frage kommenden Ligats, die folgende Schritte umfaßt:
   a) Bereitstellung einer Trägerfläche, die mit einem Hydrogel beschichtet ist, das Polyethylenoxid - Sternmoleküle mit einer Vielzahl von ligand-endständigen Polyethylenoxidketten enthält, die an einen Divinylbenzolkern gebunden sind;
   b) Kontaktierung einer ein in Frage kommendes Ligat enthaltenden Probe unter Bedingungen, die die Bindung des Ligats an den Liganden ermöglichen;

c) Entfernung aller ungebundenen Proteine von der mit Hydrogel beschichteten Fläche;
d) Einstellen der Ionenkonzentration der Probe zwecks Entfernung des gebundenen Ligats von dem Hydrogel; und
e) Sammeln der abgetrennten Ligate

12. Methode gemäß Anspruch 11, dadurch gekennzeichnet, daß die Trägerfläche aus Siliziumdioxidpartikeln, porösem, polymerem Material, polymerem Film und ultrahochmolekularem Polyethylen mit hoher Dichte ausgewählt ist.

13. Methode gemäß Anspruch 11 oder Anspruch 12, dadurch gekennzeichnet, daß das Ligat aus Makromolekülen, monoklonalen Antikörpern, Antigenen, Viren und Zellen (z. B. Blutplättchen, weißen Blutzellen und endothelialen Zellen) ausgewählt ist.

14. Methode gemäß Anspruch 11 oder Anspruch 12, dadurch gekennzeichnet, daß der Ligand aus Antikörpern (z. B. monoklonalem Antiprotein C IgG oder F_{ab} - Fragmenten von monoklonalem Antiprotein C IgG), Protein A, F_{ab}-Fragmenten von Antikörpern und aktiven Polysacchariden, z. B. Heparin, ausgewählt ist.

15. Bioverträgliches, nicht thrombogenes Hydrogel, im wesentlichen bestehend aus vernetzten Polyethylenoxid - Sternmolekülen, die auf einer Trägerfläche immobilisiert sind und eine Vielzahl von hydroxy- endständigen Polyethylenoxidketten aufweisen, die an einen polymeren Kern gebunden sind.

16. Hydrogel gemäß Anspruch 15, dadurch gekennzeichnet, daß die Sternmoleküle hydroxyendständige Polyethylenoxidketten aufweisen, die an einen polymeren Divinylbenzolkern gebunden sind, wobei das Molekulargewicht jeder Kette zwischen ca. 1.000 und ca. 10.000 liegt.

17. Kontaktlinse, die das Hydrogel gemäß Anspruch 15 aufweist.

18. Kontaktlinse gemäß Anspruch 17, dadurch gekennzeichnet, daß der polymere Kern Divinylbenzol ist und das Molekulargewicht jeder Kette zwischen 1.000 und 10.000 liegt.

Revendications

1. Procédé d'immobilisation de molécules étoilées de polyoxyde d'éthylène sur une surface de support sous la forme d'un hydrogel, comprenant les étapes consistant à:
   a) préparer une solution contenant des molécules étoilées de polyoxyde d'éthylène qui comportent une multiplicité de chaînes de polyoxyde d'éthylène terminées par des groupements hydroxy, attachées à un noyau polymère;
   b) déposer la solution sur une surface de support; et
   c) immobiliser les molécules étoilées sur la surface de support sous la forme d'un hydrogel.

2. Procédé selon la revendication 1, dans lequel l'étape c) est conduite par irradiation (par exemple avec un faisceau électronique) de la solution, pour produire un hydrogel de molécules étoilées réticulées, ladite solution étant une solution aqueuse.

3. Procédé selon la revendication 1, comprenant en outre l'étape consistant à préparer des molécules étoilées trésylées dans une solution aqueuse à un pH supérieur à 10 avant l'étape b), la surface de support contenant des groupements fonctionnels actifs (par exemple thiol, amino ou les deux), propres à immobiliser les molécules étoilées trésylées sur cette surface.

4. Procédé selon l'une quelconque des revendications 1 à 3, dans lequel le noyau polymère est un divinylbenzène.

5. Procédé selon l'une quelconque des revendications 1 à 4, dans lequel la surface de support est choisie parmi des particules, des membranes de polymère poreuses, des films de polymère, du polyéthylène haute densité de poids moléculaire ultra-élevé et des dispositifs biomédicaux.

6. Procédé selon l'une quelconque des revendications 1 à 4, dans lequel la surface de support est choisie parmi des prothèses vasculaires en contact avec le sang, des mèches angioplastiques, des fils de suture cardiovasculaire, des sondes de support métaboliques, des sondes à ballonnet angioplastiques, des

coeurs artificiels, des dispositifs d'assistance ventriculaire, des membranes d'hémodialyse et des membranes d'oxygénateurs extra-corporels.

7. Procédé selon la revendication 3, comprenant les étapes consistant à:
(i) exposer une solution organique, qui contient des molécules étoilées de polyoxyde d'éthylène comportant une multiplicité de chaînes de polyoxyde d'éthylène terminées par des groupements hydroxy, attachées à un noyau de divinylbenzène, à du chlorure de trésyle pour fixer des groupements trésyle aux groupements hydroxy terminaux;
(ii) transférer les molécules étoilées de polyoxyde d'éthylène trésylées du solvant organique à une solution aqueuse;
(iii) régler le pH de la solution aqueuse à 10 ou au-dessus; et
(iv) mettre la solution de l'étape (iii) en contact avec une surface de support contenant des groupements amino, thiol ou les deux, pour immobiliser les molécules étoilées trésylées sur cette surface, les molécules étoilées étant ainsi fixées par liaison covalente en une couche dense sur la surface de support.

8. Procédé selon la revendication 7, comprenant en outre les étapes consistant à:
a) laver la surface de support pour éliminer les éventuelles molécules étoilées non fixées, tandis que les molécules étoilées de polyoxyde d'éthylène trésylées restent fixées à cette surface; et
b) mettre la surface de support, après l'étape a), en contact avec un ligand d'affinité intéressant, comportant des groupements amino, thiol ou les deux, pour fixer le ligand aux chaînes de polyoxyde d'éthylène.

9. Procédé selon la revendication 8, dans lequel le ligand d'affinité est choisi parmi des anticorps, la protéine A, des fragments F_{ab} d'anticorps et des polysaccharides actifs (par exemple l'héparine).

10. Produit obtenu par le procédé selon l'une quelconque des revendications 1 à 9.

11. Procédé de séparation et de purification d'un ligat intéressant, comprenant les étapes consistant à:
a) préparer une surface de support sur laquelle est appliquée une couche d'un hydrogel comprenant des molécules étoilées de polyoxyde d'éthylène comportant une multiplicité de chaînes de polyoxyde d'éthylène terminées par un ligand, attachées à un noyau de divinylbenzène;
b) mettre en contact un échantillon contenant un ligat intéressant, dans des conditions appropriées pour fixer le ligat au ligand;
c) éliminer de la surface revêtue d'hydrogel les éventuelles protéines non fixées;
d) régler la force ionique de l'échantillon pour détacher ainsi le ligat fixé de l'hydrogel; et
e) recueillir les ligats qui ont été détachés.

12. Procédé selon la revendication 11, dans lequel la surface de support est choisie parmi des particules de silice, une matière polymère poreuse, un film de polymère et du polyéthylène haute densité de poids moléculaire ultra-élevé.

13. Procédé selon la revendication 11 ou 12, dans lequel le ligat est choisi parmi des macromolécules, des anticorps monoclonaux, des antigènes, des virus et des cellules (par exemple des plaquettes sanguines), des éléments blancs du sang et des cellules endothéliales.

14. Procédé selon la revendication 11 ou 12, dans lequel le ligand est choisi parmi des anticorps (par exemple l'IgG monoclonale anti-protéine C ou des fragments F_{ab} d'IgG monoclonale anti-protéine C), la protéine A, des fragments F_{ab} d'anticorps et des polysaccharides actifs (par exemple l'héparine).

15. Hydrogel biocompatible non thrombogéne, constitué essentiellement par des molécules étoilées de polyoxyde d'éthylène réticulé qui sont immobilisées sur une surface de support, lesdites molécules comportant une multiplicité de chaînes de polyoxyde d'éthylène terminées par des groupements hydroxy, attachées à un noyau polymère.

16. Hydrogel selon la revendication 15, dans lequel les molécules étoilées comprennent des chaînes de polyoxyde d'éthylène terminées par des groupements hydroxy, chaque chaîne ayant un poids moléculaire qui varie entre 1000 environ et 10 000 environ.

17. Lentille de contact, comprenant l'hydrogel selon la revendication 15.

18. Lentille de contact selon la revendication 17, dans laquelle le noyau polymère est un divinylbenzène et chaque chaîne a un poids moléculaire qui varie entre 1000 et 10 000.

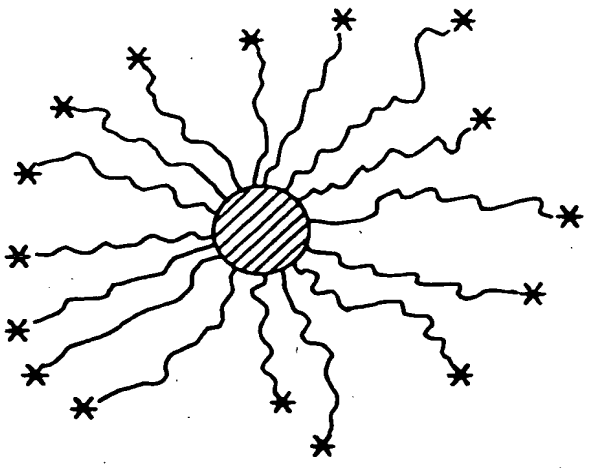


FIG. IA

LEGEND FOR FIG. IA & IB

= CROSS-LINKED DIVINYL BENZENE CORE

= PEO CHAIN

= HYDROXYL GROUP

= PS CHAIN

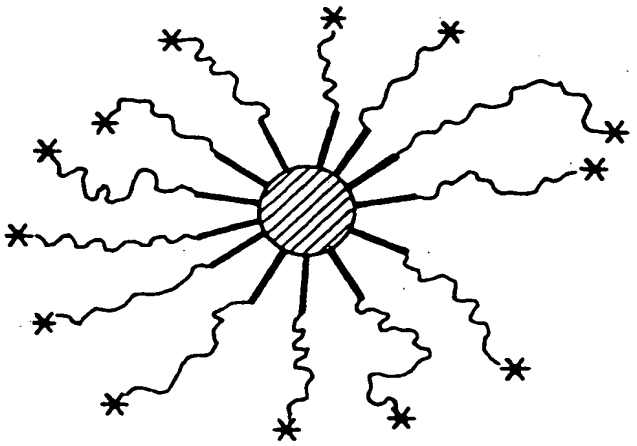


FIG. IB

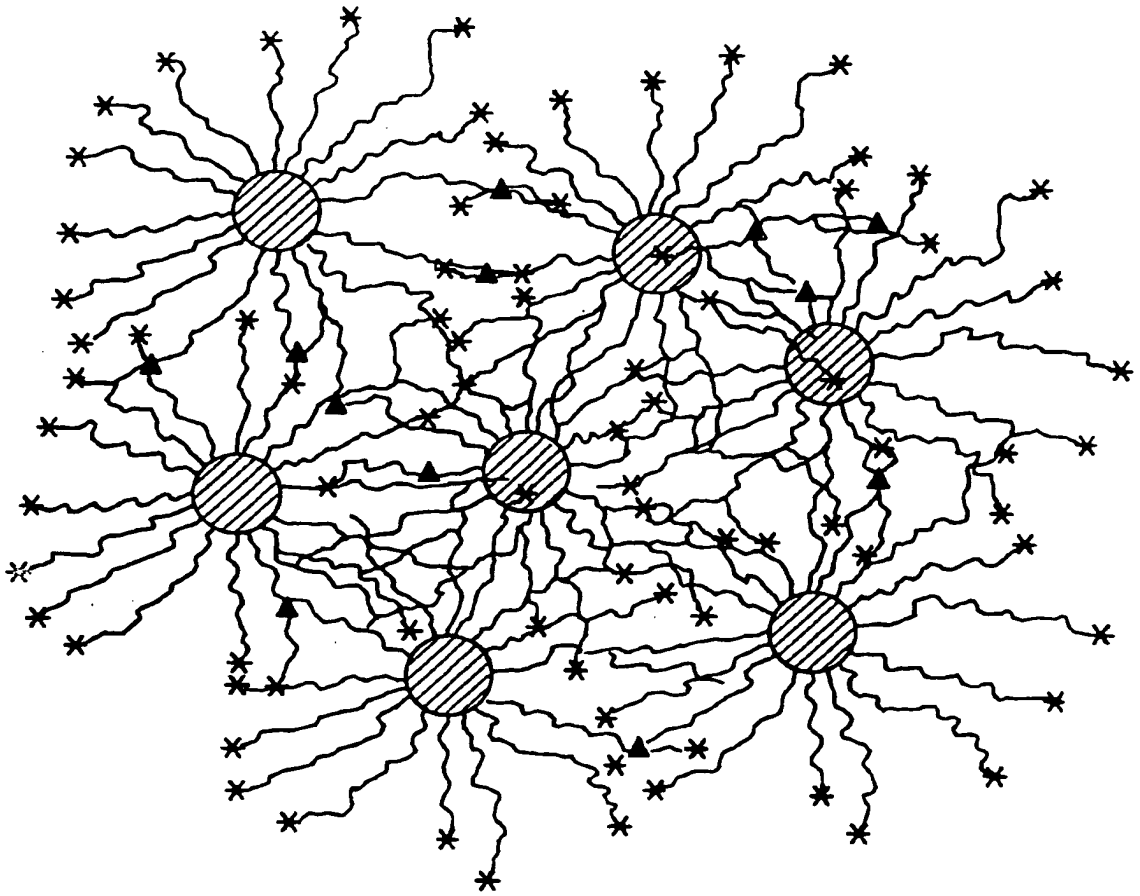
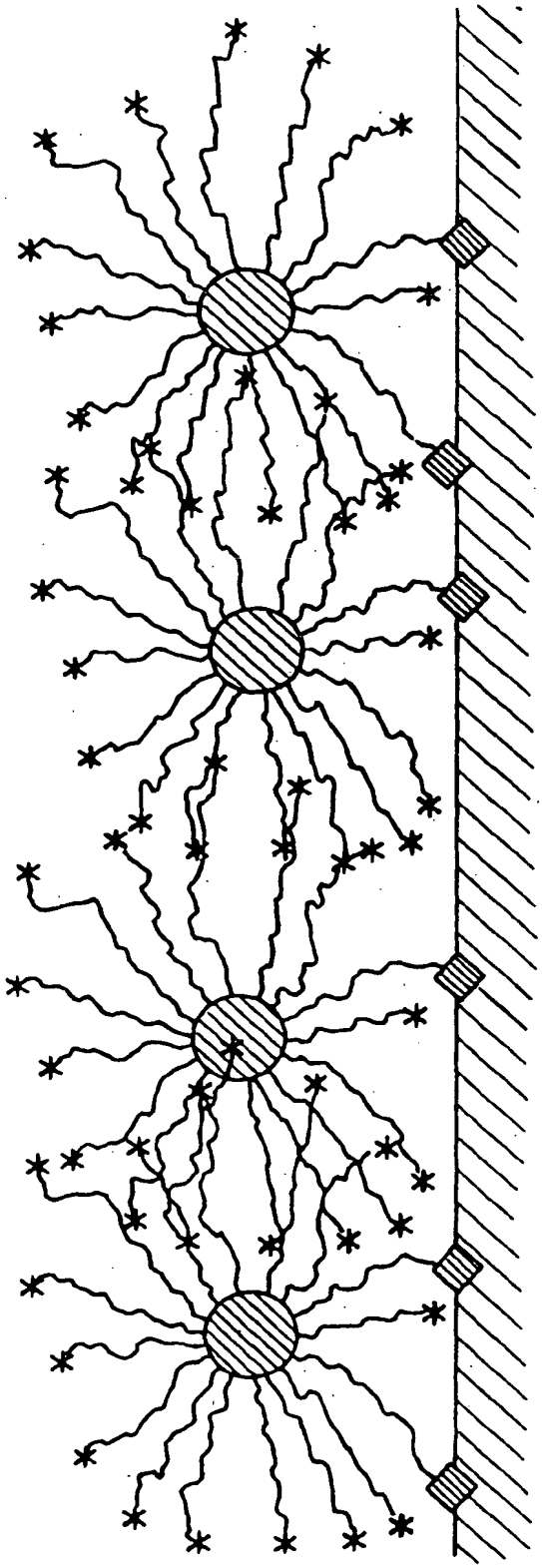


FIG. 2



* = TRESYLATED HYDROXYL

= ATTACHMENT TO AMINO GROUP ON SUPPORT SURFACE

FIG. 3

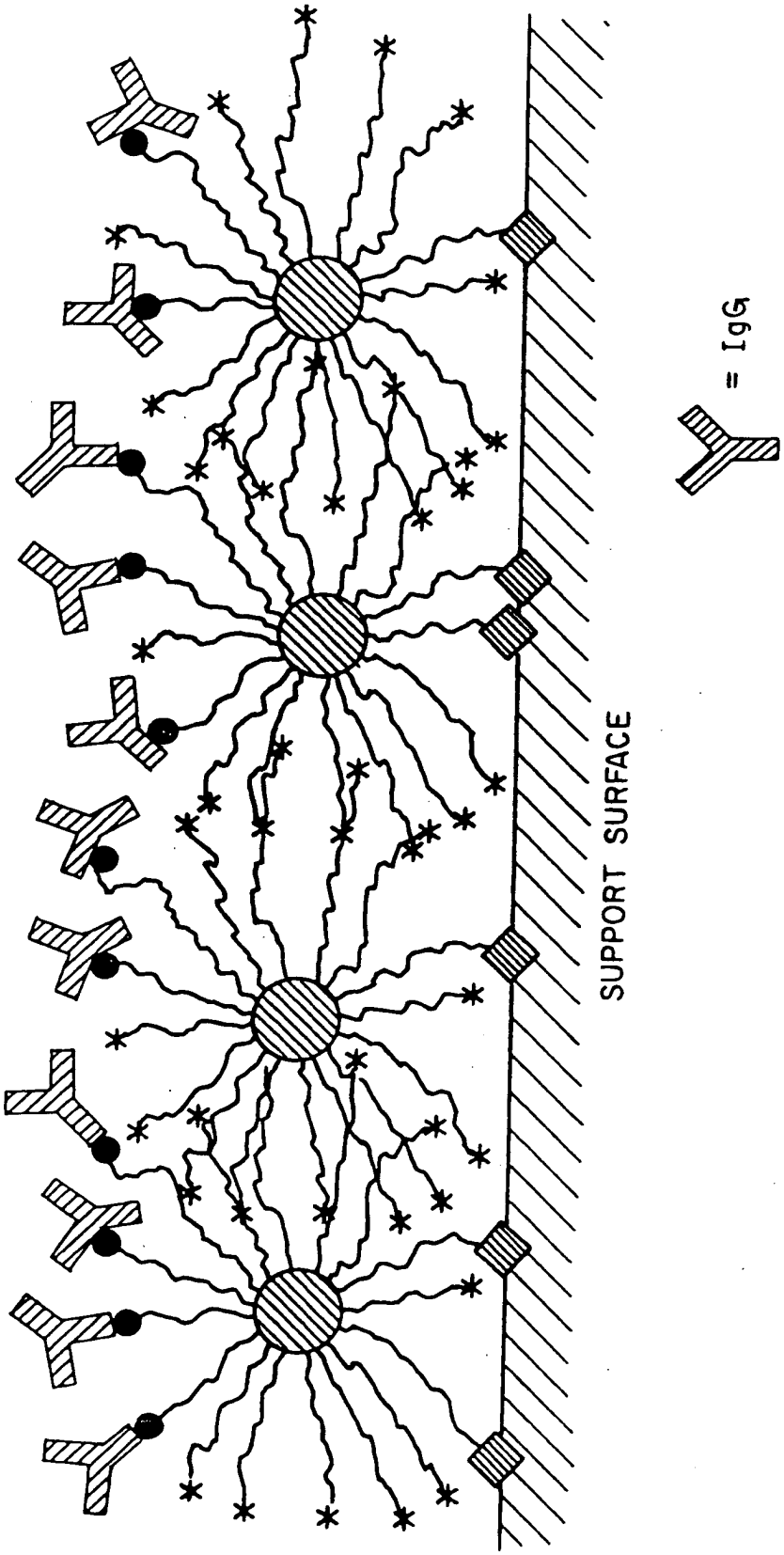


FIG. 4